

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Currently Amended) A method for identifying an immunosuppressive agent, comprising:
 - (i) providing a cell containing an NF-ATc polypeptide which is encoded by a nucleic acid that hybridizes under conditions of 5X SSC at 42 °C to SEQ ID NO:45 and has one or more of the following biological activities:
 - (a) binds calcineurin;
 - (b) undergoes nuclear localization upon T cell activation; and
 - (c) activates gene transcription upon T cell activation;
 - (ii) contacting the cell of (i) with a compound that induces nuclear translocation of the NFATc polypeptide;
 - (iii) contacting the cell before, during or after step (ii), with a test agent; and
 - (iv) assaying for nuclear translocation of the NF-ATc polypeptide, wherein an inhibition of nuclear transport in the cell relative to a cell that was not contacted with the test agent indicates that the test agent compound is a candidate immunosuppressive agent.
2. (Currently Amended) The method of claim 1, wherein the assaying for nuclear translocation comprises determining the nuclear presence of the NF-ATc polypeptide.
3. (Currently Amended) The method of claim 1, wherein the assaying for nuclear translocation comprises determining the nuclear association between the NF-ATc polypeptide and an NF-ATn polypeptide.
4. (Currently Amended) The method of claim 1, wherein the assaying for nuclear translocation comprises determining the binding of the NF-AT polypeptide or an NF-ATc:NF-ATn polypeptide complex to an NF-AT DNA binding sequence.
5. (Currently Amended) The method of claim 4, comprising using a gel mobility shift assay to determine the binding of the NF-AT polypeptide or an NF-ATc:NF-ATn polypeptide complex to an NF-AT DNA binding sequence.
6. (Currently Amended) The method of claim 1, further comprising determining the level of expression of a test nucleic acid gene linked to an NF-AT DNA binding sequence.

7. (Original) The method of claim 1, wherein the compound of step (ii) stimulates Ca^{++} release in the cell.
8. (Original) The method of claim 7, wherein the compound is ionomycin.
9. The A method of claim 1 wherein the cell further comprises an NF-ATn polypeptide and wherein assaying for nuclear translocation includes for identifying an immunosuppressive agent, comprising
 - ~~(i) providing a cell containing, or capable of expressing, NF-ATc and NF-ATn;~~
 - ~~(ii) contacting the cell of (i) with one or more compounds, such that NF-ATc and NF-ATn are present in the cell and NF-ATc is translocated into the nucleus of the cell;~~
 - ~~(iii) contacting the cell before, during or after step (ii) with a test agent;~~
 - (iv) determining the level of NF-AT NF-ATc containing complex, wherein the presence of a lower level of NF-AT complex relative to a cell that has not been contacted with a test agent indicates that the test agent is a candidate immunosuppressive agent.
10. (Currently Amended) The A method of claim 9 wherein assaying for nuclear translocation includes for identifying an immunosuppressive agent, comprising
 - ~~(i) providing a cell containing, or capable of expressing, NF-ATc and NF-ATn;~~
 - ~~(ii) contacting the cell of (i) with one or more compounds, such that NF-ATc and NF-ATn are present in the cell and NF-ATc is translocated into the nucleus of the cell;~~
 - ~~(iii) contacting the cell before, during or after step (ii) with a test agent;~~
 - (iv) ~~determining the level of NF-AT complex bound to an NF-AT binding sequence,~~ wherein the presence of a lower level of bound NF-AT complex relative to that in a cell that has not been contacted with the test agent indicates that the test agent is a candidate immunosuppressive agent.
11. (Currently Amended) A method for identifying an immunosuppressive agent, comprising
 - (i) contacting a purified NF-ATc polypeptide or cell extract containing an NF-ATc polypeptide with a purified NF-ATn polypeptide, or a cell extract containing an NF-ATn polypeptide and a test agent, under conditions which permit the formation of an NF-AT complex, wherein the NF-ATc polypeptide is encoded by

by a nucleic acid that hybridizes under conditions of 5X SSC at 42 °C to SEQ ID NO:45 and has one or more of the following biological activities:

(a) binds calcineurin;

(b) undergoes nuclear localization upon T cell activation; and

(c) activates gene transcription upon T cell activation; and

- (ii) determining the level of NF-AT complex formed, wherein a lower level of NF-AT complex relative to the level of NF-AT complex formation in the absence of the test agent indicates that the test agent is a candidate immunosuppressive agent.
12. (Currently Amended) The method of claim 11, wherein the NF-ATc or NF-ATn polypeptide is immobilized.
13. (Currently Amended) The A method of claim 1 wherein the cell further includes—for identifying an immunosuppressive agent comprising
- (i) ~~—providing a cell containing, or capable of expressing, NF-ATc and NF-ATn and further containing an NF-AT regulated enhancer region linked to a test nucleic acid gene; and assaying for nuclear translocation includes~~
- (ii) ~~—contacting the cell of (i) with one or more compounds, such that NF-ATc and NF-ATn are present in the cell and NF-ATc is translocated into the nucleus of the cell;~~
- (iii) ~~—contacting the cell before, during or after step (ii) with a test agent; and~~
- (iv) ~~—determining the level of expression of the test gene, wherein a lower level of expression of the test gene relative to its level of expression in a cell that was not contacted with the test agent indicates that the test agent compound is a candidate immunosuppressive agent.~~
14. (Currently Amended) The method of claim ~~15~~ 13, wherein the test gene encodes a protein which is essential for cell proliferation or viability.
15. (Currently Amended) The A method of claim 1 wherein the cell further includes—for identifying an immunosuppressive agent comprising
- (i) ~~—providing a cell containing, or capable of expressing, NF-ATc and NF-ATn and further containing an NF-AT regulated enhancer region linked to a test nucleic acid gene; and assaying for nuclear translocation includes~~
- (ii) ~~—contacting the cell of (i) with one or more compounds, such that NF-ATc and NF-ATn are present in the cell and NF-ATc is translocated into the nucleus of the cell;~~

- (iii) ~~contacting the cell before, during or after step (ii) with a test agent; and~~
- (iii) determining the level of expression of the test gene, wherein a higher level of expression of the test gene relative to its level of expression in a cell that was not contacted with the test agent indicates that the test agent compound is a candidate immunostimulatory agent.
16. (Original) The method of claim 15, wherein the test gene encodes a protein which is essential for cell proliferation or induces cell death.
17. (Currently Amended) A method for identifying an immune regulating agent, comprising contacting a cell or a cell extract containing an NF-ATc polypeptide with a test agent compound, wherein the NF-ATc polypeptide is encoded by a nucleic acid that hybridizes under conditions of 5X SSC at 42 °C to SEQ ID NO:45 and has one or more of the following biological activities:
- (a) binds calcineurin;
 - (b) undergoes nuclear localization upon T cell activation; and
 - (c) activates gene transcription upon T cell activation; and
- (i) determining the level of phosphorylation of the NF-ATc polypeptide, wherein a difference in the level of phosphorylation relative to that of a cell or cell extract that was not contacted with the test agent indicates that the test agent is a candidate immune regulating agent.
18. (Currently Amended) The method of claim 17, further comprising contacting the cell with an agent which induces the nuclear translocation of the NF-ATc polypeptide ~~NF-ATc~~.
19. (Currently Amended) A method of any one of claims 1, 9, 10, 13, 15, and 17, wherein the NF-ATc polypeptide is encoded by a heterologous nucleic acid in the cell.
20. (Original) A method of claim 19, wherein the NF-ATc polypeptide or portion thereof comprises at least 25 amino acids having an amino acid sequence which is substantially identical to an amino acid sequence set forth in SEQ ID NO:46.
21. (Original) A method of claim 19, wherein the NF-ATc polypeptide or portion thereof is encoded a nucleic acid which hybridizes to a nucleic acid having the nucleotide sequence set forth in SEQ ID NO:45 or the complement thereof.
22. (Withdrawn) A method for diagnosing the immune status of a subject, comprising determining the presence, amount, and/or location of an NF-ATc polypeptide in T cells

of the subject, wherein the presence of a pathognomic amount, or staining pattern in the T cells indicates the presence of a hypofunctional or hyperfunctional T cell condition or a predisposition to develop a disease.

23. (Withdrawn) A method for monitoring the level of an immunosuppressant in the blood of a subject comprising determining the presence, amount, and/or location of an NF-ATc polypeptide in T cells of the subject, wherein the presence of a pathognomic amount, or staining pattern in the T cells of the subject is indicative of the level of immunosuppressant in the blood of the subject.
24. (Withdrawn) A method for modulating an immune response in a subject, comprising administering to the subject a therapeutically effective dose of a pharmaceutical composition comprising an agent identified by any one of claims 1, 9, 10, 11, 13, 15, and 17.